

## Application of GC-MS/MS for the Analysis of Anabolic Steroids in Meat Products

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### Abstract

This application note illustrates the utility of gas chromatography coupled to tandem quadrupole mass

spectrometry (GC-MS/MS) in addressing these requirements, particularly when compared with MS<sup>1</sup> low resolution measurements (LRMS) and High Resolution Mass Spectrometry (HRMS).

## Benefits

- Offers maximum confirmation for GC amenable compounds

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## Introduction

Natural and synthetic hormones have been used worldwide for many years to improve rates of protein deposition in livestock. Although steroids or steroid-like compounds are widely used and licensed in various countries, such applications are prohibited in the European Union. In spite of the official ban, a black market demand for hormones and hormone cocktails does exist. Policing against the illicit use is carried out under the auspices of the EU directive 96/23/EC1 and implemented through surveillance under the National Plan of individual member states. For controls to be effective at the stall level as well as for imported products into the EU, development of efficient detection methods in meat was necessary. To monitor biological specimens and establish the presence of anabolic agents and their metabolites, it is essential to have analytical techniques for detecting trace amounts of these compounds. The list of steroids liable to be abused in cattle fattening is ever lengthening and the analytical requirements are increasingly stringent. For this reason, specific immunoassays that allow for the detection of a single molecule are now being replaced by multi-residue techniques such as mass spectrometry combined with chromatographic separations. In addition to identifying steroids historically permitted in select countries (e.g. melengestrol acetate, trenbolone acetate, zeranol, progesterone, estradiol, testosterone and their esters), analytical methods must guarantee the low ppt (pg/g) detection of the anabolics most often associated with illegal usage (nandrolone, methyltestosterone, norethandrolone, medroxyprogesterone, or megestrol). As more fields focused on steroid analysis at ultra-trace level emerge (e.g. the study on endocrine disruptors in water supplies) methods must become more sensitive (pg level), more adaptable (e.g. to matrices such as hair, urine, faeces, water), more specific (high-resolution measurement, bidimensional MS acquisition) and more effective with challenging analytes such as endogenous steroids (isotopic composition determination).

This application note illustrates the utility of gas chromatography coupled to tandem quadrupole mass spectrometry (GC-MS/MS) in addressing these requirements, particularly when compared with MS<sup>1</sup> low resolution measurements (LRMS) and High Resolution Mass Spectrometry (HRMS).

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## Experimental

### Reagents and Chemicals

All solvents and reagents were of analytical and HPLC grade quality. All SPE (C<sub>18</sub>, SiOH, NH<sub>2</sub>, SiOH, SCX cartridges) were single use cartridges. Purified *Helix pomatia* preparation was used for steroid deconjugation to avoid any side effects due to interfering isomerase and oxidoreductase.

Trimethylsilylating reagent (TMS) and N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) were purchased from Fluka (Buchs, Switzerland). Dithiothreitol (DTT) and ammonium iodide (NH<sub>4</sub>I) were from Aldrich (Milwaukee, WI, USA). Standard reference steroids were purchased from Sigma (St Louis, MO, USA), Steraloids (Wilton, NY, USA), Research Plus (Bayonne, NJ, USA).

### Sample Preparation

The analytical protocols dedicated to the extraction and purification of anabolic steroids in urine and edible tissue have been described elsewhere.<sup>2,3,4</sup>

### Instrumentation

For GC-MS experiments, an HP-6890 gas chromatograph was coupled to an HP-5973 (Agilent Technologies, Palo-Alto, USA) quadrupole mass spectrometer (low resolution single MS), as well as to a SX102A (Jeol, Tokyo, Japan) double focusing electromagnetic instrument (high resolution MS).

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## Results and Discussion

### Trenbolone in Urine, MSTFA-I2 derivatization

Derivatized trenbolone was first analyzed by GC-MS, where to enable confirmation using low resolution single quadrupole selected ion recording (SIR) it is necessary to acquire a minimum of four ions. A blank urine was analysed, followed by a spiked urine, spiked at 1.5 µg L<sup>-1</sup>. Figure 1 shows traces for the blank urine and spiked urine analyzed by GC-MS and Figure 2 shows the traces for blank urine and spiked urine analyzed by GC-MS/MS. It can be clearly seen that although GC-MS is easily capable of detecting the target peaks, the selectivity of MS/MS gives a much better signal to noise ratio for the target peaks, giving the analyst much greater confidence in results obtained.

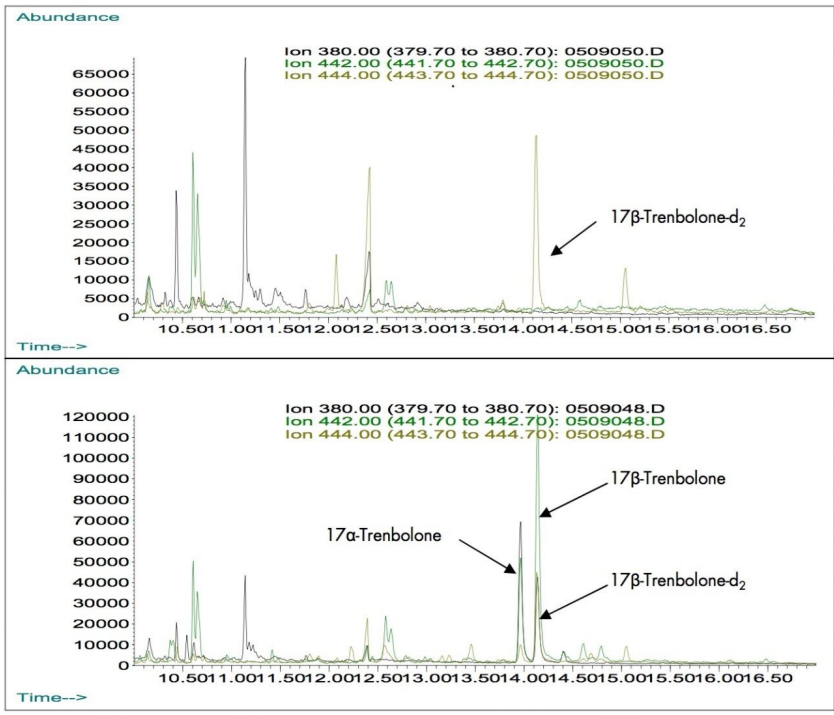


Figure 1. Blank (upper trace) and 1.5 µg L<sup>-1</sup> spiked (lower trace) trenbolone in urine, GC-MS.

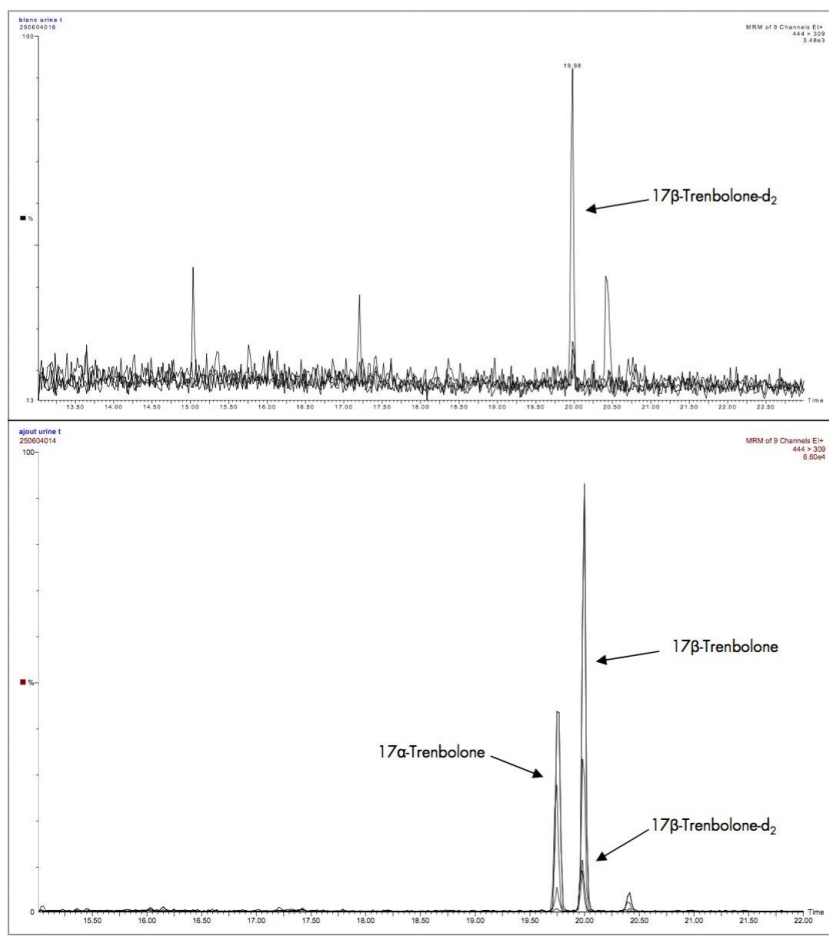


Figure 2. Blank (upper trace) and  $1.5 \mu\text{g L}^{-1}$  spiked (lower trace) trenbolone in urine, GC-MS/MS.

## Estrogens in Water

Derivatized estrogens were analyzed at a  $1\text{pg ml}^{-1}$  (1 ppt) spike level in water using GC-MS/MS and GC-HRMS (10,000 resolution). The chromatograms for the analysis by GC-MS/MS are shown in Figure 3, and the overlaid chromatograms for the GC/HRMS analysis are shown in Figure 4. The difference in the signal to noise ratio between GC-MS/MS and GC-HRMS is not very significant, and may not justify the additional cost of a magnetic sector mass spectrometer.

Because the estrogen masses used for the GC-HRMS analysis are mass sufficient, they are not very well resolved from the background chemical noise (as is the case for dioxin/PCB analysis, where the target compounds are mass deficient). However, in the case of GC-MS/MS, the selectivity of MS/MS clearly resolves target compounds from the chemical background, resulting in the achievement of good LODs.



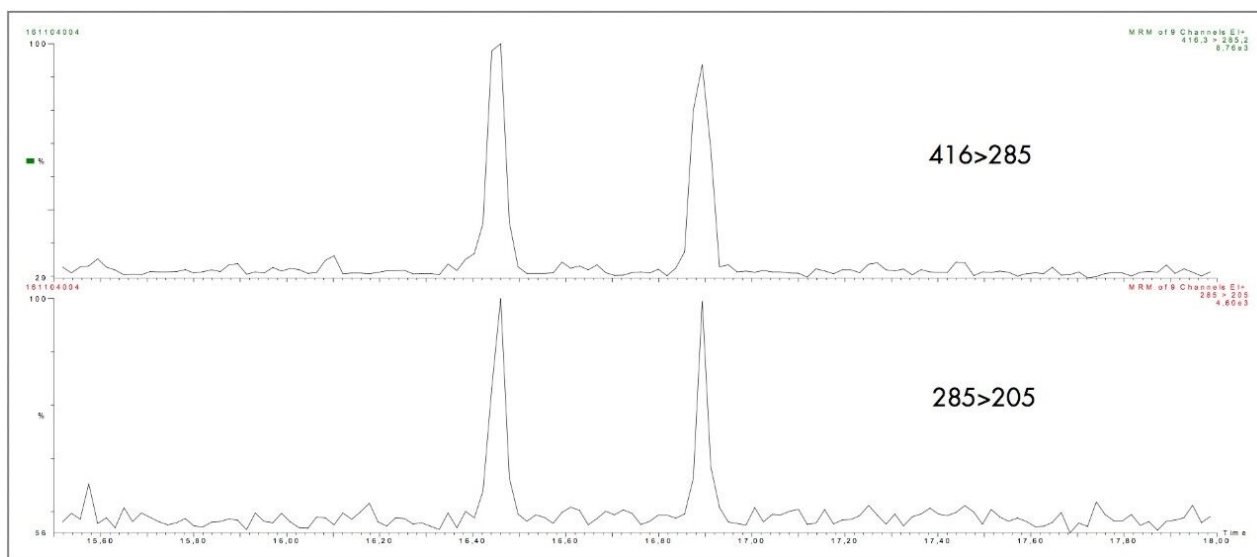


Figure 3.  $0.001 \mu\text{g L}^{-1}$  estrogens in water, GC-MS/MS.

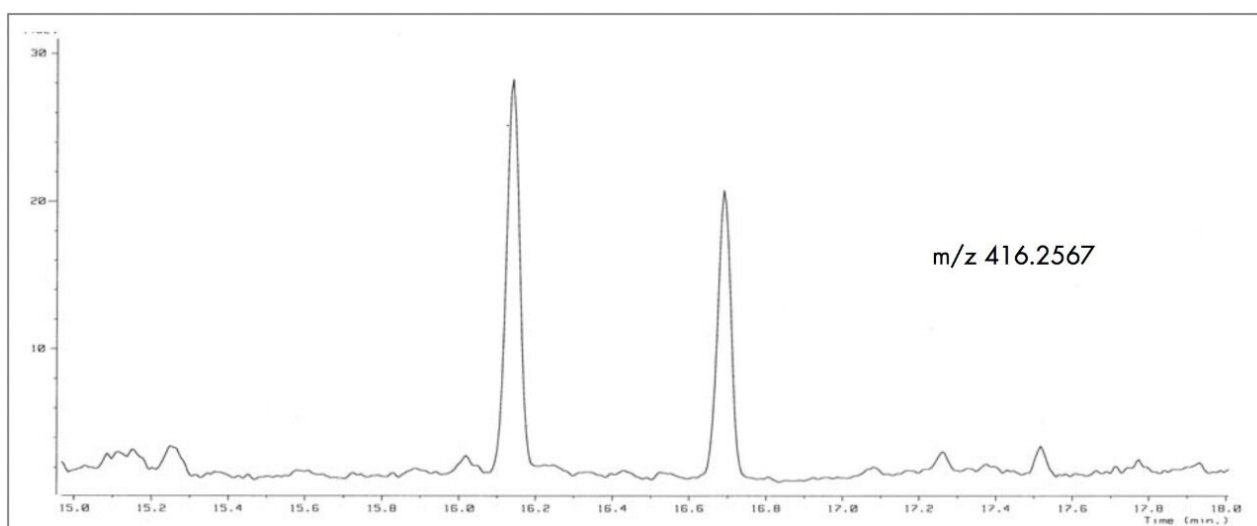


Figure 4.  $0.001 \mu\text{g L}^{-1}$  estrogens in water, GC/HRMS.

## Androgens in Meat

Figure 5 shows the chromatograms for Androstendione spiked into water (upper 2 traces,  $0.05 \mu\text{g kg}^{-1}$ ) and meat (lower 2 traces,  $0.04 \mu\text{g kg}^{-1}$ ). The chromatograms illustrate very good selectivity for the target compound with comparable signal to noise (taking the concentration difference into account) between the two matrices.

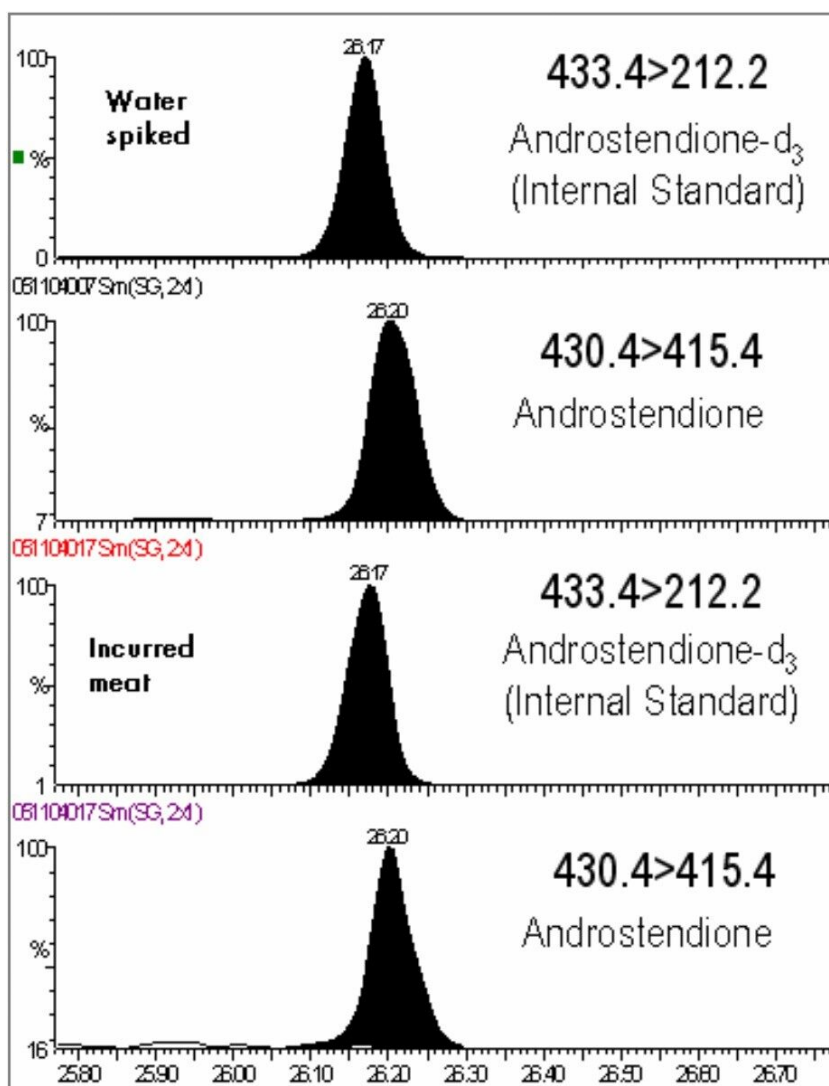


Figure 5. Androstendione and androstendione-d<sub>3</sub> spiked into water (upper traces) and meat (lower traces).

Reviewing large datasets and ensuring that all EU confirmatory criteria are satisfied can be a time consuming process when performed manually; the possibility of user error also increases. With Waters TargetLynx Application Manager, an option with Waters MassLynx Software for automated processing and reporting of MS data, the user is able to rapidly review a data set for any nonconformances.

Figure 6 shows a dataset where the testosterone coefficient of determination  $r^2$  falls outside the tolerance specified in the method, and as a result, all testosterone injections are clearly highlighted with a red background.

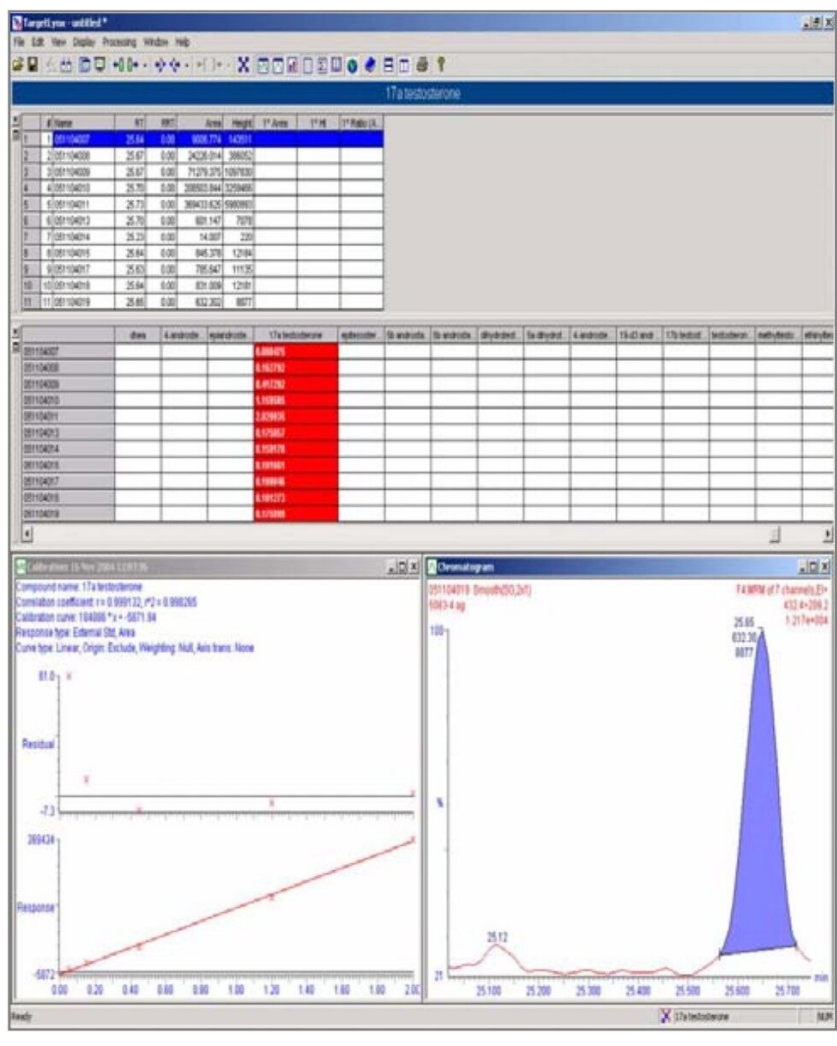


Figure 6. TargetLynx browser view.

## Conclusion

The analysis of anabolic steroids in meat products can be performed by a variety of techniques with differing degrees of confirmation. To achieve the maximum confirmation for GC amenable compounds, the use of GC-MS/MS is now a requirement for the laboratory analyst. The results presented clearly show the power of GC-MS/MS for the confirmatory analysis of trace components in complex matrices.



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## References

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720001764, May 2006